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Evaluation of phytochemical, anti-oxidant and cardiac depressant effect of *Rumex dentatus* by using Langendorff's isolated heart apparatus

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Abstract: *Rumex dentatus* has been used traditionally for ailment of cardiovascular diseases. The aim of the present study was to assess cardiovascular effects in isolated perfused rabbit heart. Aqueous and n-butanolic fractions were assessed for their effect on perfusion pressure (PP), force of contraction (FC) and heart rate (HR) of rabbit heart using Langendorff's method. The possible mechanisms of action of extracts/fraction were assessed with and without application of different agonist/antagonist. Phytochemical, toxicity and anti-oxidant activities were also determined. Both extracts at 1mg/mL dose produced a highly significant decrease in FC and HR but PP remained unchanged. Moreover, aqueous fraction of *Rumex dentatus* at 0.001mg/mL dose produced a highly significant decrease in FC and HR but no significant change in PP was observed. Atropine 10^{-5} M did not inhibit the cardiac depressant response of both fractions. Furthermore, both fractions blocked the positive ionotropic and chronotropic effects of adrenaline and calcium chloride. Phytochemical studies have shown the presence of some phytochemicals. Acute and sub-chronic toxicity studies demonstrated that test extracts are safe and produced no significant changes in haematological and biochemical parameters. Crude extract showed significant antioxidant activity like ascorbic acid. This study revealed that this plant have good cardiac depressant effect.

Keyword: *Rumex dentatus*, phytochemical, anti-oxidant, cardiac depressant, Langendorff's method, animal models.

INTRODUCTION

Medicinal plants have constantly remained a major source for drug in human history. Significant number of the medicines used currently are of medicinal plant source. In developing countries, medicinal plants used as a substitute therapy, due to its cost effectiveness and easy availability. It has been projected that 1/3rd globally humans depend on traditional drugs for their treatment. Although traditional medicines are effective, however it need to be investigated on scientific basis (Ali & Qaiser, 2009; Thomford *et al*., 2018). In the developing world, cardiovascular diseases responsible for morbidity and mortality due to lifestyle (Tabuti *et al.,* 2003). Some examples of medicinal plants which are reported effective against the hypertension and cardiovascular diseases are *Rauwolfia serpentine* and *Allium sativum* etc. (Weng *et al.,* 1984; Suter *et al.,* 2006).

Rumex dentatus is a species of flowering plant and it belong to the family of Polygonaceae. It is known by the common Pushto language name "Jungle Sag". It is annual or biennial herb and native to different parts of Asia and North Africa, and it is also located outside distributional range. It is also located in Punjab and Khyber Pakhtunkhwa (KPK) provinces of Pakistan (Badshah & Hussain, 2011). Traditionally *Rumex dentatus* is used as diuretic, astringent, purgative and analgesic (Manandhar, 2002; Fatima *et al.,* 2009). *Rumex dentatus* is reported for antibacterial, antifungal, insecticidal, molluscicidal, anti-inflammatory, dermatitis and allelopathic activity (Litvinenko & MuzychKina, 2003; Hussain *et al.,* 2010; Nisa *et al*., 2013). However, *Rumex dentatus* is not reported yet for its cardiac depressant activity. This study explored the cardiovascular effects of the crude extract and fractions of *Rumex dentatus* on the isolated rabbit heart.

MATERIALS AND METHODS

Chemicals and drugs

Methanol, n-butanol, atropine, adrenaline and calcium chloride were purchased from Sigma-Aldrich, USA.

Experimental animals

Rabbits (1-1.5kg), Sprague Dawley (SD) rats (200-

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280gm) and Albino mice (30-40gm) of either gender were used. Standard housing conditions were provided to all the experimental animals in the animal house at University of Sargodha, Sargodha, Pakistan. The experimental procedure was approved by the institutional ethical committee and National Research Council (NRC, 1996).

Preparation of plant extract

Rumex dentatus was collected in the month of November from district Sargodha, Pakistan. The plant and was identified and verified by Taxonomist, Dr. Amin-u-Allah, Assistant Professor, Botany department, University of Sargodha, Pakistan. After that aqueous methanolic (30:70) extract were prepared from the 3kg leaves of *Rumex dentatus* by using cold maceration method. This crude extract was stored in a cool place.

Fractionation of crude extract

The procedure of Rehaman *et al*. (2015) was followed to prepare the n-butanolic and aqueous fractions of *Rumex dentatus.*

Experimental procedures

Acute toxicity test

The protocols of Shetty *et al*. 2007 and Falya *et al*. 2020 were followed with some changes to find out the acute toxicity of *Rumex dentatus.* For this purpose the mice are randomly divide into five groups; Control group, received normal saline, while groups two to five received 500, 1000, 1500 and 2000 mg/kg extract at intra-peritoneal (i.p) route respectively. The animals were observed for 24 hours.

Sub-chronic toxicity

The protocol of Moulisha *et al.* 2010 was followed with some modification to find out the sub-chronic toxicity of *Rumex dentatus* (500 mg/kg) in SD rats. The blood samples of all experimental rats were collected at the end of experimental period (28 days) for biochemical parameters analysis.

Effect of Rumex dentatus extract and its fractions on isolated rabbit heart

The experiments were carried out according to the protocol established by Langendorff's (1985). The isolated rabbit heart was used to study the effect of *Rumex dentatus* on the HR (beats/min), FC (g) and PP (mm Hg) by using Langendorff's isolated heart apparatus. To find out the possible mechanism (s), the response of the n-butanolic and aqueous fractions of *Rumex dentatus* (10ng, 100ng, 1µg, 10µg, 100µg/mL) were investigated in the presence of atropine (10^{-5} M; muscarinic receptors antagonist), adrenaline (10^{-5} M; adrenergic receptors agonist) and calcium chloride (10^{-5} M) (Farid *et al.* 1992; Shah *et al.,* 2010; Abdullah *et al.,* 2012).

Determination of antioxidant potential of crude extract of Rumex dentatus

The antioxidant activity of the crude extract of *Rumex dentatus* was measured according to the protocol followed by Sreejayan *et al*. 1996. The different concentrations of crude extract used in this protocol were 5, 10, 25, 50 and 100µg/mL, respectively.

Phytochemical screening

The procedure of Edeoga *et al*. (2005) was followed to identify the different phytochemical constituents in the extract of *Rumex dentatus.*

STATISTICAL ANALYSIS

The data obtained are presented as means ± SEM. Student's t-test and two way ANOVA followed Bonferroni's test, using GraphPad Prism Software, version 8 (Graph Pad, San Diego, CA, USA). $P<0.05$ was considered as significant.

RESULTS

Acute toxicity study

This study confirmed that the crude extract of *Rumex dentatus* is safe up to 1500 mg/kg. LD_{50} of the crude extract of *Rumex dentatus* was found to be 2000 mg/kg in rats. No signs of toxicity were observed in the dose range from 500 to 1500 mg/kg. The allergic reaction, irregular heart rate and changes in breathing was also not revealed within the safe range of dosing.

Sub-chronic toxicity study

The sub-chronic study results confirmed that crude extract of *Rumex dentatus* produced no significant signs of toxicity. A single dose of 500 mg/kg/p.o of *Rumex dentatus* did not show any mortality. Moreover, the hematological, body internal body organs weight, and serum biochemical (LFTs, lipid profile) values were also not altered significantly in comparison to control values table 1, fig.1.

Effect of crude extract, n-butanolic and aqueous fraction fraction of Rumex dentatus on various cardiac parameters

The crude extract of *Rumex dentatus*, in the doses from 0.00001mg/mL to 1mg/mL indicates a significant increase in PP. However, the crude extract exhibited a highly significant ($P<0.05$) decrease in the HR and FC at 1mg/mL dose. The maximum response in PP, HR and FC was observed at 1mg/mL (fig. 2A). The n-butanolic fraction of *Rumex dentatus*, in the doses from 0.00001 mg/mL to 1mg/mL exhibited a significant increase in PP. However, the n-butanolic fraction shows a highly significant decrease in the HR and FC at 1mg/mL. The maximum response in all the three parameters was observed at 1mg/mL. Therefore the dose of 1mg/mL was

Table 1: Complete blood count of rats

WBC(X1000)		RBC(106/mm3)		HBg/dl		HCT(PCV)%		MCV fl		Platelets(X1000)	
C	T	C	T	C	T	C	T	C	T	C	T
9.6±1.4	10.0±0.5	7.1± 0.1	8.68± 0.27	11.8± 0.09	12.3± 0.72	42.0± 1.15	49.7± 0.8*	53.0± 1.7	55.7± 1.7	821 ± 6.90	920 ± 7.50**

Neutrophils %		Lymphocytes %		Monocytes %	
C	T	C	T	C	T
11.6±0.30	10.5±0.29	67.7±0.88	69.3±2.40	1.5±0.2	2.4±0.29*

Key: Results are expressed as Means ± S.E.M (n = 3) (C= Control, T= Treated), Where ** = $p < 0.01$ and * = $p < 0.05$ vs. normal control.

Table 2: Phytochemical screening

S. No	Phytochemicals constituents	Observations
1	Alkaloids	+
2	Indole alkaloids	+
3	Tannins	+
4	Phenols	+
5	Flavonoids	+
6	Saponins	+
7	Cardiac glycosides	-
8	Steroids	+
9	Terpenoids	+

Key: Present: +, Absent: -

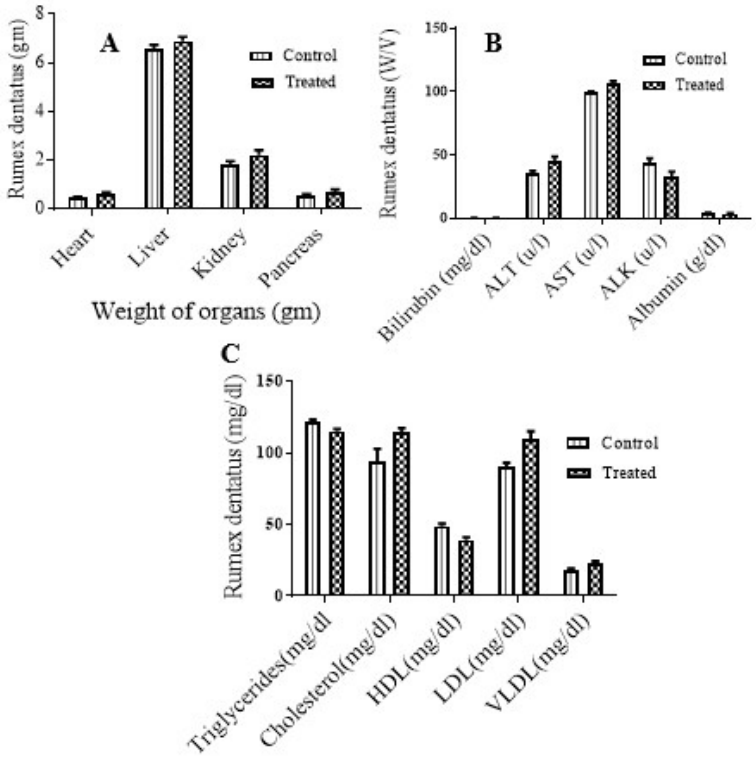

Fig. 1: Bar graphs shows the effects of crude extract of *Rumex dentatus* on (A) different organs (B) liver enzymes and (C) lipid profile of rats.

chosen for the determination of possible mechanism of action (fig. 2B). Furthermore, the aqueous fraction of *Rumex dentatus*, in concentration from 0.00001mg/mL to 1 mg/mL exhibited a significant increase in PP. However, the aqueous fraction exhibited a highly significant decrease in the FC and HR at 0.001mg/mL. Therefore the dose of 0.001mg/mL was chosen for the determination of possible mechanism of action (fig. 2C).

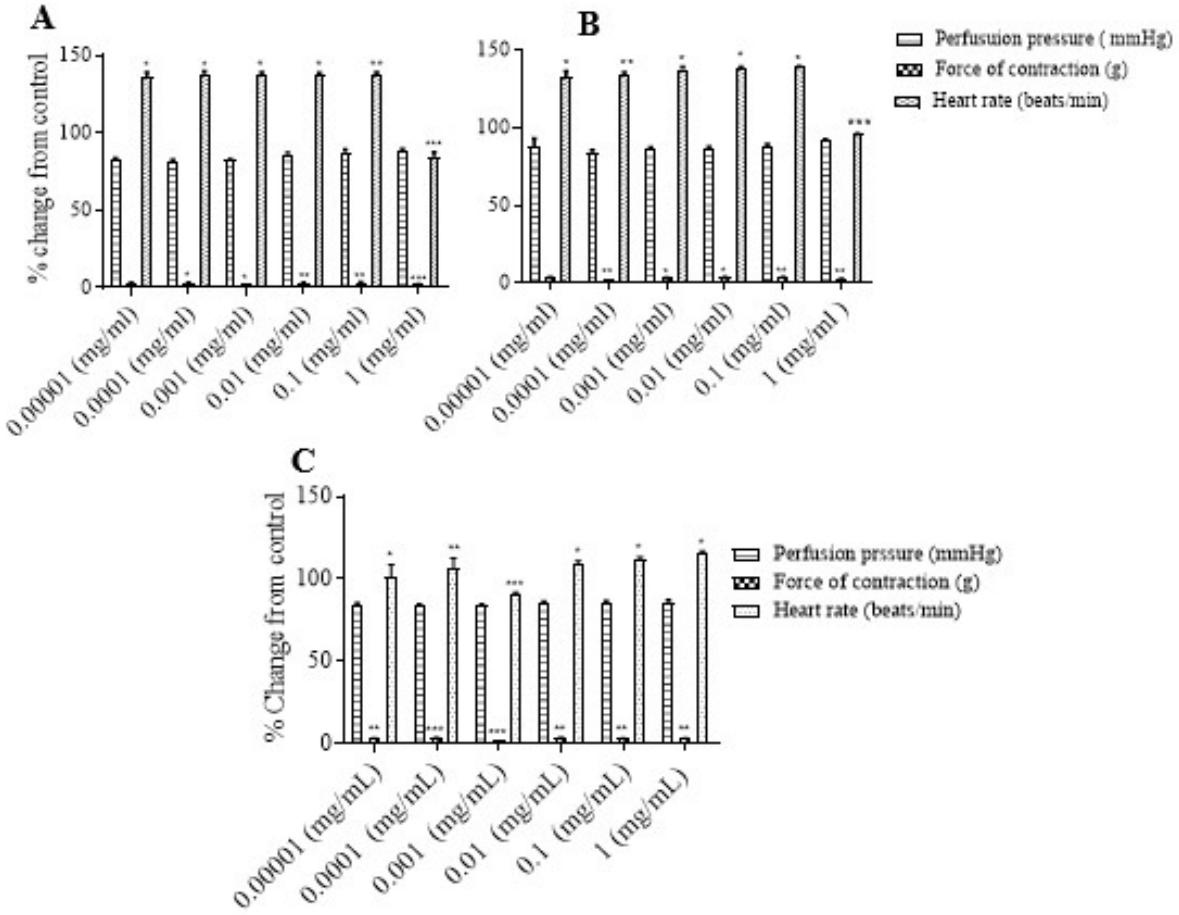

Fig. 2: Effect of different concentration of (A) crude extract, (B) n-butanolic fraction and (C) aqueous fraction of *Rumex dentatus* on various cardiac parameters; perfusion pressure, force of contraction and heart rate on rabbit isolated heart. Whereas *** = P<0.0001, ** =P<0.01 and * =P<0.05 as compared to control (n=6).

Determination of anti-muscarinic, β-adrenergic and calcium channel blocking activity of n-butanolic and aqueous fractions of Rumex dentatusIn the presence of atropine 10^{-5} M, the n-butanolic fraction at a dose of 1mg/mL and aqueous fraction 0.01mg/mL shows non-significant decrease in FC and HR. Moreover, a non-significant change was also observed in PP (fig. 3). The effects of adrenaline 10^{-5} M and calcium chloride10^{-5} M were significantly reduced in the presence of n-butanolic and aqueous fractions. Adrenaline and calcium chloride produces a highly significant change in FC and HR in the absence of fractions. However, a prominent increase in PP was observed by adrenaline and calcium chloride both in the presence and absence of the fractions (fig. 3).

Antioxidant activity of Rumex dentatus crude extract
In this study, the DPPH scavenging activity of the aqueous methanolic extract of *Rumex dentatus* was compared with reference compound, ascorbic acid. The assay performed reveals significant antioxidant potential in comparison to ascorbic acid (fig. 4).

Phytochemical screening
The phytochemical screening of the *Rumex dentatus* confirmed the presence of various phytochemicals constituents such as polyphenols, flavonoids, saponinsm tannins, steroids, terpeniods, alkaloids and indole alkaloids. The presence of cardiac glycosides was not observed table 1.

DISCUSSION

The acute toxicity study confirmed that the *Rumex dentatus* extract was safe up to the dose of 1500mg/kg in mice, which shows the high safety level of *Rumex dentatus*. The zero mortality rate in sub-chronic toxicity study also confirmed the safety profile of *Rumex dentatus*. Moreover, no significant changes in the hematological parameters, LFTs and lipid profile confirmed that the *Rumex dentatus* extract is safe, although a rise (within the safe range) in few parameters (platelets, monocytes and HCT) was observed in comparison to control.

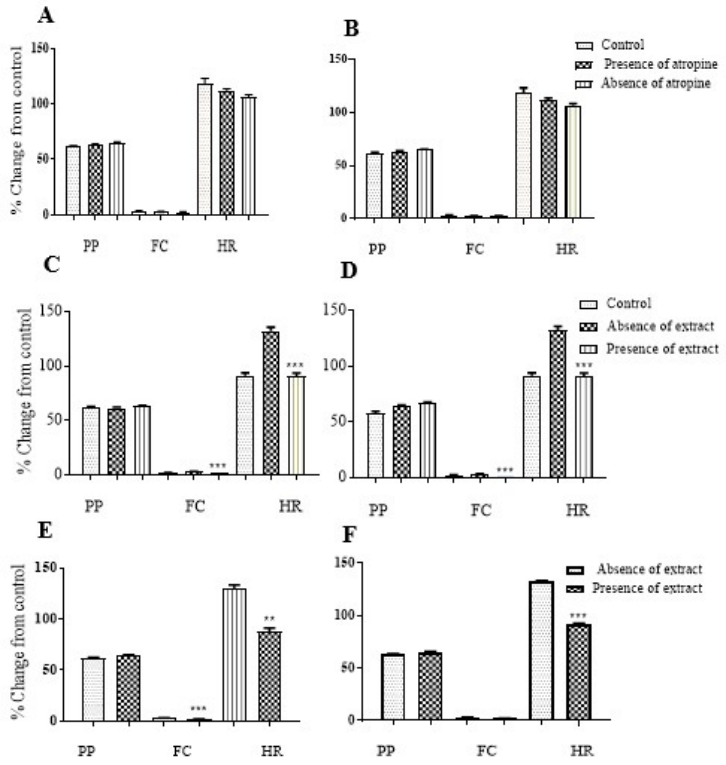

Fig. 3: The bar graphs shows the effects of atropine 10^{-5} M (A, B), adrenaline 10^{-5} M (C, D) and calcium chloride 10^{-5} M (E, F) in presence and absence of n-butanolic (1mg/mL) (A, C, E) and aqueous fraction (0.001mg/mL) (B, D, F) of *Rumex dentatus* on PP, FC and HR of isolated rabbit heart. Where ** = P<0.001 and *** =P<0.0001 as compared to control.

Our findings of *in-vitro* experiments on isolated rabbit heart exhibited that *Rumex dentatus* extract produce a decrease in FC and HR. In present study it was noted that crude extract and n-butanolic fraction of *Rumex dentatus* at different concentrations show increase in PP and reduce the FC and HR, however, both the fraction and extract at concentration of 1mg/mL show maximum decrease in FC and HR. The most significant concentration, 1mg/mL of n-butanolic fraction and 0.001mg/mL of aqueous fraction were selected for the determination of underlying mechanism (s). This study has confirmed that cardiac depressant response of both fractions of *Rumex dentatus* might be due to the vasorelaxant effect or decrease in myocardial contractility. Our results have shown that both n-butanolic and aqueous fractions of extract of *Rumex dentatus* did not act through muscarinic receptors because atropine 10^{-5} M did not block the cardiac depressant effects of extract.

To confirm the involvement of of β-adrenergic receptors, adrenaline was used. Adrenaline is an adrenergic agonist drug and acts through a and b adrenergic receptors to increase the heart rate and contraction of heart (Hartung *et al.,* 1990). The result shows that both fractions of *Rumex dentatus* extract block the response of adrenaline 10^{-5}M indicated the connection of β-adrenergic receptors. Furthermore, calcium chloride was used to confirm the involvement of calcium channels. In this study contractile response of calcium chloride 10^{-5} M also blocked by both fractions of *Rumex dentatus* extract. As phytochemical screening confirmed the presence of different phytochemicals. Flavonoids might be responsible for cardiac depressant response of *Rumex dentatus* as reported previously (Umang *et al.,* 2009). Flavonoids are reported for diuretic, anti-inflammatory and vasoprotective activities. Other phytochemical like alkaloids, tannins and saponins are reported for cardiac depressant, diuretic and vasodilatory effects (Sankari *et al*., 2014).

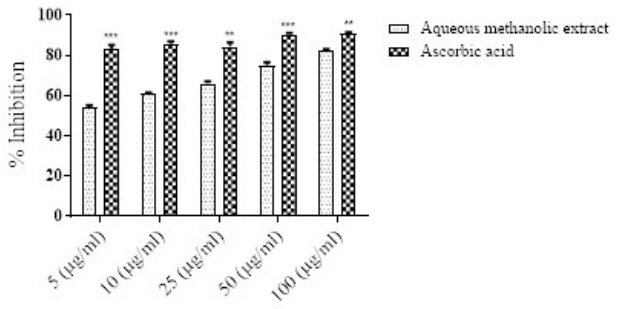

Fig. 4: Different concentration of *Rumex dentatus* extract, showing the antioxidant activity. Values are expressed as mean ± S. D. (n=6). ***p<0.0001 and **p<0.001m shows significant values.

Some studies have reported that antioxidant activity may reduce the pathological changes lead to rise in blood pressure and cardiovascular disorders. Furthermore, it has been hypothesized that there might be a correlation between oxidative stress and cardiovascular disorders (Boshtam *et al*., 2002). So, we also study the antioxidant effect of *Rumex dentatus.* Antioxidant activity of crude extract was investigated through DPPH method. DPPH is a stable organic free radical that has been extensively used to determine antioxidant activity of many natural products (Sowndhararajan & Kang, 2013). The findings exhibited a significant antioxidant potential of the crude extract of *Rumex dentatus*. However in comparison, the antioxidant potential of *Rumex dentatus* is less than ascorbic acid.

Study limitation and future research

The performed phytochemical analysis is very primitive and further investigation could be incorporated (due to limitation of funding and instruments). Analysis of the plant extract by LC/MS is strongly recommended for full identification of active ingredient. Separation of extract using preparative HPLC for isolation of active principles and further repeating of cardiovascular activity for each active ingredient should be performed to identify which compound is responsible for the reported cardiovascular activity.

CONCLUSION

In conclusion, the various bioactive compounds in the extract and fractions of *Rumex dentatus* may be exert their cardiac depressant effects by different mechanisms, including blockage of β-adrenergic receptors and calcium channels. Further investigation of phytochemicals present in the extract will elaborate the mentioned findings.

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